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AB - PURPOSE: To investigate whether dendritic cells (DC), which as professional antigen presenting cells have the capacity to stimulate immune responses against tumour associated antigens, are recruited into and activated within prostate cancer. MATERIALS AND METHODS: Immunoenzyme and immunofluorescence labelling was used to identify leucocyte and DC subsets within 15 cases of prostate cancer. Cell numbers were compared with numbers in adjacent normal prostatic tissue. Total DC numbers were identified as CD45+ leucocytes not coexpressing any lineage specific markers. The Langerhans cell (LC) subset was detected using anti CD1a staining and activated DC were identified by their expression of either CD83, CD86 or CMRF44. RESULTS: DC were found to represent a small subset of leucocytes present in both benign and malignant prostatic tissue. Statistically there were significantly less DC and LC in prostate cancer compared with normal prostatic tissue. While only a small subset of DC expressed markers of activation in prostate cancer, this was significantly more than the virtual absence of activated DC in normal prostatic tissue. CONCLUSIONS: This is the first time that DC have been studied in prostate cancer using the relatively new DC specific monoclonal antibodies CD83 and CMRF-44. These findings suggest that there is no active recruitment of DC into prostate cancer and those DC present are only minimally activated.  
AD - Haematology/Immunology Research Group, the Department of Urology, Christchurch Hospital, New Zealand.  
FAU - Troy, A  
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MH - Humans  
MH - Leukocytes/immunology  
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## A Novel Immunological Model for the Study of Prostate Cancer<sup>1</sup>

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### Abstract

The Dunning R-3327 rat prostatic adenocarcinoma is a widely accepted model for *in vivo* experimental studies of prostate cancer. We have previously derived phenotypically distinct cell lines from a s.c. tumor resulting from the inoculation of the R-3327-5 subclone into Copenhagen rats. In this study, we report studies using a gelatin sponge model for the delivery of tumor cells and the retrieval of tumor-specific leukocytes responsive to different prostatic cell lines. S.c. preimplanted sponges were inoculated with tumor cells previously selected for differential properties of tumor formation and metastasis and examined for leukocyte content at time points of 1, 3, and 5 weeks after tumor cell inoculation. Cytospin and flow cytometric analyses revealed fewer tumor-associated leukocytes present in sponges inoculated with tumorigenic R-3327-5' and R-3327-5'B lines, with lesser sponge degradation, than in experiments with the nontumorigenic R-3327-5'A line, suggestive of a tumor cell-induced immunomodulatory mechanism. Morphological studies indicate an intermittent tumor growth pattern that gradually disappears in sponges inoculated with the nontumorigenic R-3327-5'A cells but a robust growth pattern in sponges inoculated with the tumorigenic cell lines. Cytokine analyses show the secretion of higher levels of active transforming growth factor- $\beta$  by the more invasive and metastatic lines. Total transforming growth factor- $\beta$  levels are higher in the epithelial, tumorigenic R-3327-5'B line. Additionally, the more tumorigenic lines secrete interleukin 10, a potent immunosuppressive molecule. In this report, we demonstrate the ability to retrieve viable leukocyte populations from a prostate tumor line bearing sponges, which offers an important model for further *in vitro* and *in vivo* manipulations and holds promise for testing adoptive immunotherapeutic strategies.

### Introduction

The Dunning R-3327 Copenhagen rat model of prostate cancer is a well-established experimental system, having been initially described in 1963 (1). Original cell lines developed from cultures of the primary tumor have been maintained by serial transplantation in animals and have yielded numerous phenotypically diverse sublines, owing to their inherent genetic instability. These cell lines have proven invaluable as experimental tools in prostate cancer research because they can be manipulated in fully immunocompetent animals (2). However, the cellular heterogeneity demonstrated by these cell lines poses a significant challenge in understanding the invasive and metastatic phenotypes of different cells within a given cultured line. To circumvent this biological complexity, we isolated clonal cell lines, R-3327-5'A and R-3327-5'B, from a heterogeneous parental tumor arising after s.c. injections of R-3327-5 cells into male rats (3). Whereas R-3327-5'B cells grow in epithelial-like clusters, produce large primary tumors

subsequent to s.c. injection, and are poorly metastatic and invasive, R-3327-5'A cells maintain a fibroblast-like phenotype, do not produce a primary tumor, and are highly metastatic and invasive. The parental cell line exhibits both epithelial and fibroblastic phenotypes found in the clonal lines, produces a significant primary tumor, and is moderately metastatic and invasive. These cell lines thus provide unique tools for studying potential differences in the host/tumor immunological response elicited by prostate cancer cells expressing an epithelial, fibroblastic, or combined phenotype.

Recruitment and infiltration of host immune cells in a developing tumor is a widely accepted biological response. Although both CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes have been shown to be important players in tumor progression and regression (4, 5), identifying the role of other immunological cells and factors in the developing tumor mass has become an important challenge. From these and other studies, it has emerged that various tumor cells can interact actively with their microenvironment and induce immunomodulatory responses (6). For example, TGF- $\beta$  and IL-10 are two factors that have been implicated in tumor-induced immunosuppressive responses (6-9). Experimental strategies for examining these interactions in transplantable tumor systems have primarily involved s.c. injections of single-cell suspensions and subsequent examination of the solid tumor mass. Possible loss of tumor cells from the inoculation site, as well as the inability to effectively characterize tumor growth as attributable to infiltrating host cells or tumor inoculum, are both inherent drawbacks of such an approach. To address this issue, Park *et al.* (10) developed a retrievable gelatin sponge model for studying tumor growth and isolating infiltrating leukocytes responding to different mouse mammary carcinoma cell lines. We have adapted this sponge model technology for studying and characterizing the variation in immunological response(s) to the R-3327-5'-derived prostate tumor subpopulations. The ability to use the sponge retrieval system with our newly developed prostate cell lines cloned from a common parental population provides a unique opportunity to test the hypothesis that a weak immunological response is associated with prostate cells that form primary tumors and conversely, that a strong immunological response is associated with prostate cancer cells that do not form primary tumors. These data may provide important clues into the diverse immune responses elicited by aggressive versus nonaggressive prostatic disease, thereby leading to better therapeutic strategies.

### Materials and Methods

**Tumor Cell Lines.** The Dunning R-3327-5' and derived clones R-3327-5'A and R-3327-5'B rat prostatic adenocarcinoma lines have been described previously and characterized by our laboratory (3). Briefly, the R-3327-5' line was established from a primary culture of a s.c. rat tumor formed in response to injection of R-3327-5 cells. The clonal lines, R-3327-5'A (fibroblastic phenotype) and R-3327-5'B (epithelial phenotype), were generated by single

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<sup>3</sup> The abbreviations used are: TGF, transforming growth factor; IL, interleukin; NK, natural killer; PMN, polymorphonuclear; TNF, tumor necrosis factor.

cell isolation of morphologically distinct cells from the primary culture (see Fig. 1). The cell lines were maintained and propagated *in vitro* by serial passage in RPMI 1640 medium supplemented with 15% fetal bovine serum (Life Technologies, Inc., Grand Island, NY), 2 mM L-glutamine (Life Technologies, Inc.), 1 mM sodium pyruvate (Sigma Chemical Co., St. Louis, MO), 10 mM HEPES (Life Technologies, Inc.), 100 units/ml penicillin-streptomycin sulfate (Life Technologies, Inc.), and 0.2% gentamicin (Sigma).

**In Vivo Studies.** Eight to 12-week-old male Copenhagen rats (Harlan Sprague Dawley, Indianapolis, IN) were used in this study. All animals were housed in The University of Iowa Animal Care Facility, according to the NIH Health Guide for the Care and Use of Laboratory Animals. All protocols were reviewed and approved by the Institutional Animal Care and Use Committees.

**Sponge Implantation and Tumor Cell Inoculation.** The experimental methodology for this study is outlined in Fig. 1. The surgical procedure was a modification of that described previously by Akporiaye *et al.* (11). Sterile gelatin sponges (Gelfoam; Upjohn Co., Kalamazoo, MI) were trimmed to approximately  $10 \times 10$ -mm squares and hydrated in sterile, pyrogen free PBS. The dorsal surfaces of the rats were shaved and swabbed thoroughly with Betadine solution (Purdue Frederick, Norwalk, CT) prior to surgery. One sponge was surgically implanted s.c. into each shoulder (four sponges/animal) of ketamine (91 mg/kg body weight)/xylazine (9.1 mg/kg body weight) anesthetized rats. Sponge implantation was performed 7 days before injection of tumor cells (referenced as day 1).

Monolayer cultures of Dunning R-3327-5', clone R-3327-5'A and R-3327-5'B cells at ~70% confluence were enzymatically removed from tissue culture

plastic, washed, and resuspended to a concentration of  $2.5 \times 10^6$  cells/ml in sterile PBS. Subsequently,  $2.5 \times 10^5$  cells (in 0.1 ml) were injected s.c. into each of two randomly selected sponges per animal. The remaining two sponges were injected with sterile PBS and served as controls in the same animals, thereby eliminating the need to account for individual variability with respect to the immune response.

**Sponge Retrieval and Cell Isolation.** To retrieve the implanted sponges, animals were anesthetized with Metofane and sacrificed by cervical dislocation at 1, 3, and 5 weeks (days 15, 29, and 43 after sponge implantation, respectively) after tumor cell inoculation. Visible tumors at the site of sponge implantation and tumor cell injection were measured using a caliper and compared with sites containing control sponges. Sponges from animals in similar experimental or control groups were surgically excised, pooled, and disaggregated using a modification of the method described previously by Akporiaye *et al.* (11). Briefly, pooled sponges were minced in a beaker containing 20 ml of a prewarmed enzyme mixture consisting of Saline G (composition in g/l: 1.1 g glucose, 8.0 g of NaCl, 0.4 g of KCl, 0.29 g of  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 0.15 g of  $\text{KH}_2\text{PO}_4$ , 0.005 g of phenol red, 0.15 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.016 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) supplemented with 500 mg of BSA (Sigma), 30 mg of collagenase IV (222 units/mg; Worthington Biochemicals, Freehold, NJ), and 200  $\mu\text{l}$  of 2% DNase (Sigma). The minced sponge suspension was digested by gentle shaking at  $37^\circ\text{C}$  for 2 h. The resulting cell suspensions were filtered through 350- and 140- $\mu\text{m}$  polypropylene meshes (Spectrum, Houston, TX) and supplemented with 10 ml of culture medium.

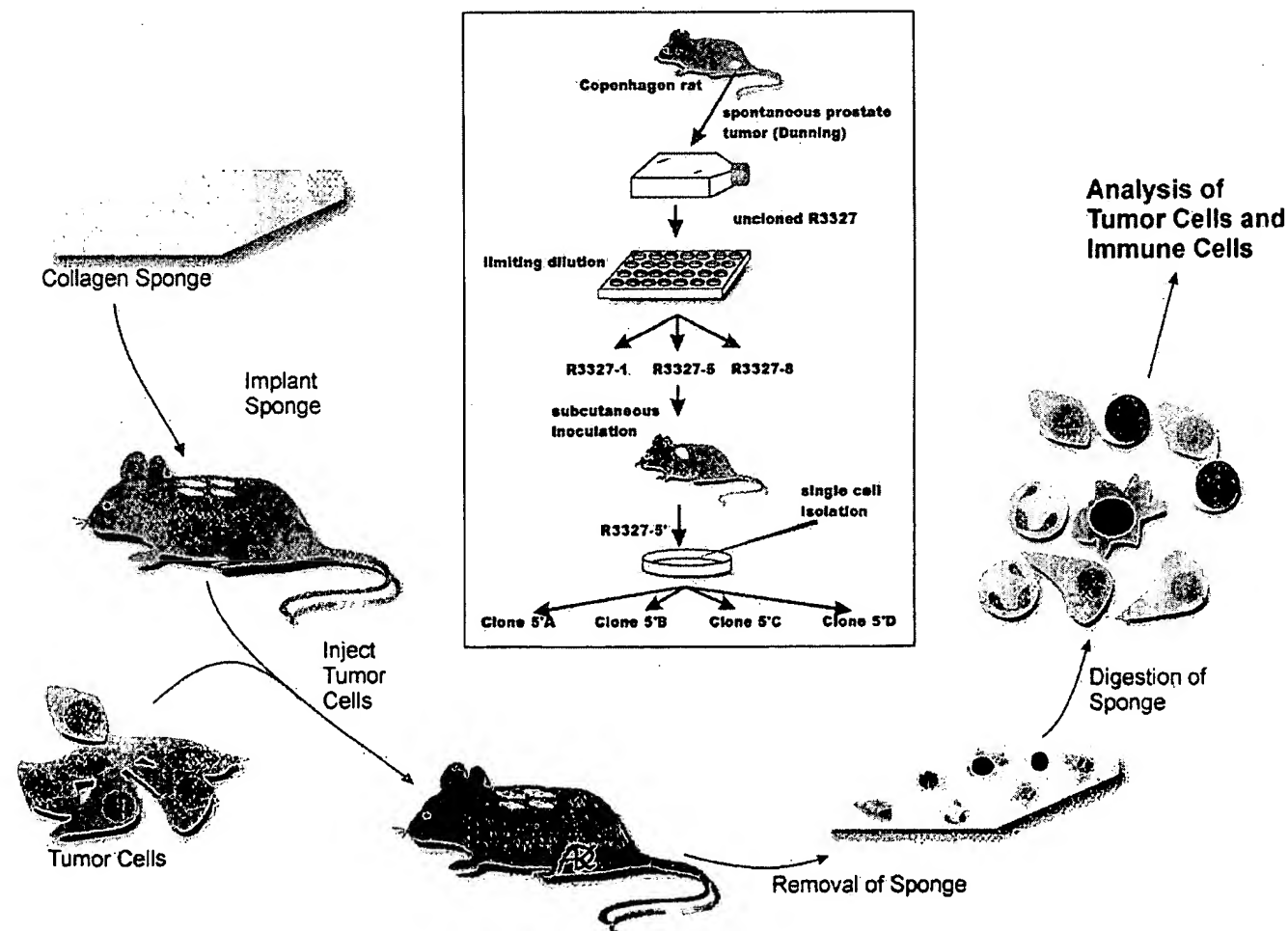
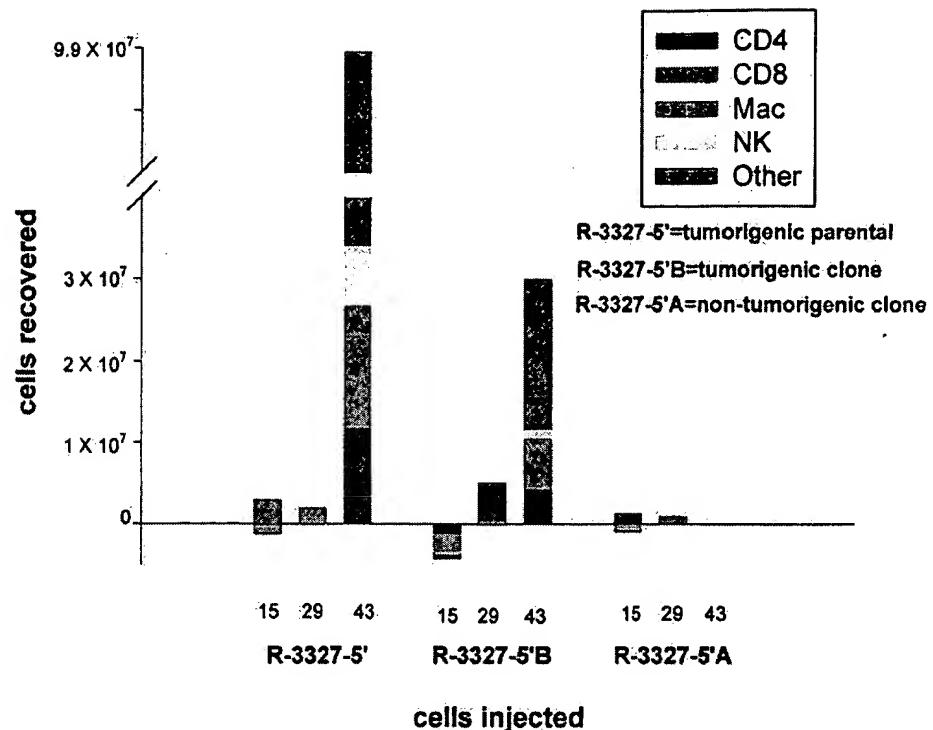


Fig. 1. Experimental methodology flow chart of the gelatin tumor-sponge system. R-3327-5, a poorly invasive subline of the original spontaneous rat prostate tumor, was s.c. injected into the right flank of male Copenhagen rats, and single cell clones were picked from the resultant heterogeneous tumor to yield four distinct clones. Clone R-3327-5'B, a highly tumorigenic, poorly invasive epithelial-like cell line, and R-3327-5'A, a nontumorigenic, highly invasive fibroblast-like cell line, together with the parental heterogeneous R-3327-5', were tested for their ability to elicit an immune response using the gelatin sponge model of tumor-associated leukocyte retrieval. Rats, 8–12 weeks of age, were implanted s.c. with four sterile gelatin sponges each. One week later,  $2 \times 10^5$  Dunning R-3327-5', 5'A, or 5'B cells were injected directly into two of the preimplanted sponges, whereas the other two served as controls. Sponges were removed at time points of 1, 3, and 5 weeks after inoculation, digested in collagenase, and analyzed for tumor growth patterns and leukocyte content.

Fig. 2. Leukocyte profile of sponge-retrieved cells. Disaggregated sponge cells were analyzed for total cell counts using a hemacytometer and characterized for leukocyte content by labeling for CD4<sup>+</sup>, CD8<sup>+</sup>, macrophage (MAC), and NK cell populations. Cells were harvested for analyses on days 15, 29, and 43. Control (medium-injected) cell numbers from the same animal were subtracted from those in the experimental pool. Data presented below the x-axis represent lower leukocyte and other cell numbers (compared with controls) collected from tumor cell-inoculated sponges.



**Determination of Cell Viability and Total Cell Number.** A 100- $\mu$ l aliquot of the digested cell suspension was stained with 2  $\mu$ l of propidium iodide (1 mg/ml in PBS) and 1  $\mu$ l of acridine orange (1 mg/ml in PBS), and cells were assessed using a hemacytometer and a fluorescence microscope equipped with the appropriate filters (11). Acridine orange imparts a green nuclear fluorescence to viable cells, whereas propidium iodide imparts a red fluorescence to the nuclei of dead cells. Erythrocytes, lacking nuclei, do not interfere with the analysis. Cell viability was ~90% throughout the duration of these experiments.

**Immunological Characterization of Sponge-infiltrating Cells.** All cellular manipulations were performed on ice. Cell suspensions (isolated from sponges) were adjusted to a concentration of  $3 \times 10^6$  cells/ml, and 0.1 ml of these suspensions was used for cellular characterization by antibody labeling. Cells were resuspended and blocked for 30 min in RPMI 1640 containing 2% BSA before incubation with monoclonal antibodies to rat CD4, CD8, NKR-PIA (a natural killer cell marker) and an ED-2-like macrophage antigen (PharMingen, San Diego, CA), and diluted 1:100 in the RPMI-BSA blocking medium. After extensive washing, cells were incubated with a FITC-conjugated goat anti-mouse secondary antibody (Cappel, West Chester, PA), diluted 1:200 in blocking medium. The labeled cells were then washed, fixed in 3.7% formaldehyde in PBS, and then resuspended in PBS. Control samples consisted of unlabeled cells, as well as those incubated with only the secondary antibody. All flow cytometric analyses were performed at The University of Iowa Flow Cytometry Facility. Autofluorescence was minimal, and nonspecific secondary antibody binding was always within acceptable limits. Forward/orthogonal scatter gates were set to exclude tumor cells. Gates were also set to eliminate debris and aggregates from consideration.

**Morphological Studies.** For cytological analysis, 80  $\mu$ l of each tumor-cell suspension were cytocentrifuged onto glass microscopic slides and stained using the Leukostat kit (Fisher Scientific, Pittsburgh, PA). Stained slides were examined and photographed using a Nikon FotoFX microscope fitted with a Nikon FX 35DX camera.

Scanning electron microscopic analysis was performed by fixing the sponges in 2.5% glutaraldehyde in 0.1 M sodium cacodylate immediately after excision. Fixed specimens were processed and analyzed at The University of Iowa Central Electron Microscopy Research Facility, as described previously (12).

**Cytokine Analysis.** R-3327-5', R-3327-5'A and R-3327-5'B were assayed for *in vitro* production of immunomodulatory cytokines using the Riboquant

RNase protection assay system (PharMingen). Briefly, total RNA was extracted from cultured cells and hybridized with the radiolabeled rCK-1 multi-probe template set, which assays for IL-1, IL-3, IL-4, IL-5, IL-6, IL-10, IFN- $\gamma$ , and tumor necrosis factors  $\alpha$  and  $\beta$  (PharMingen). Hybridized and nonhybridized RNA was then treated with RNase, and the hybridized probes were visualized on a polyacrylamide sequencing gel.

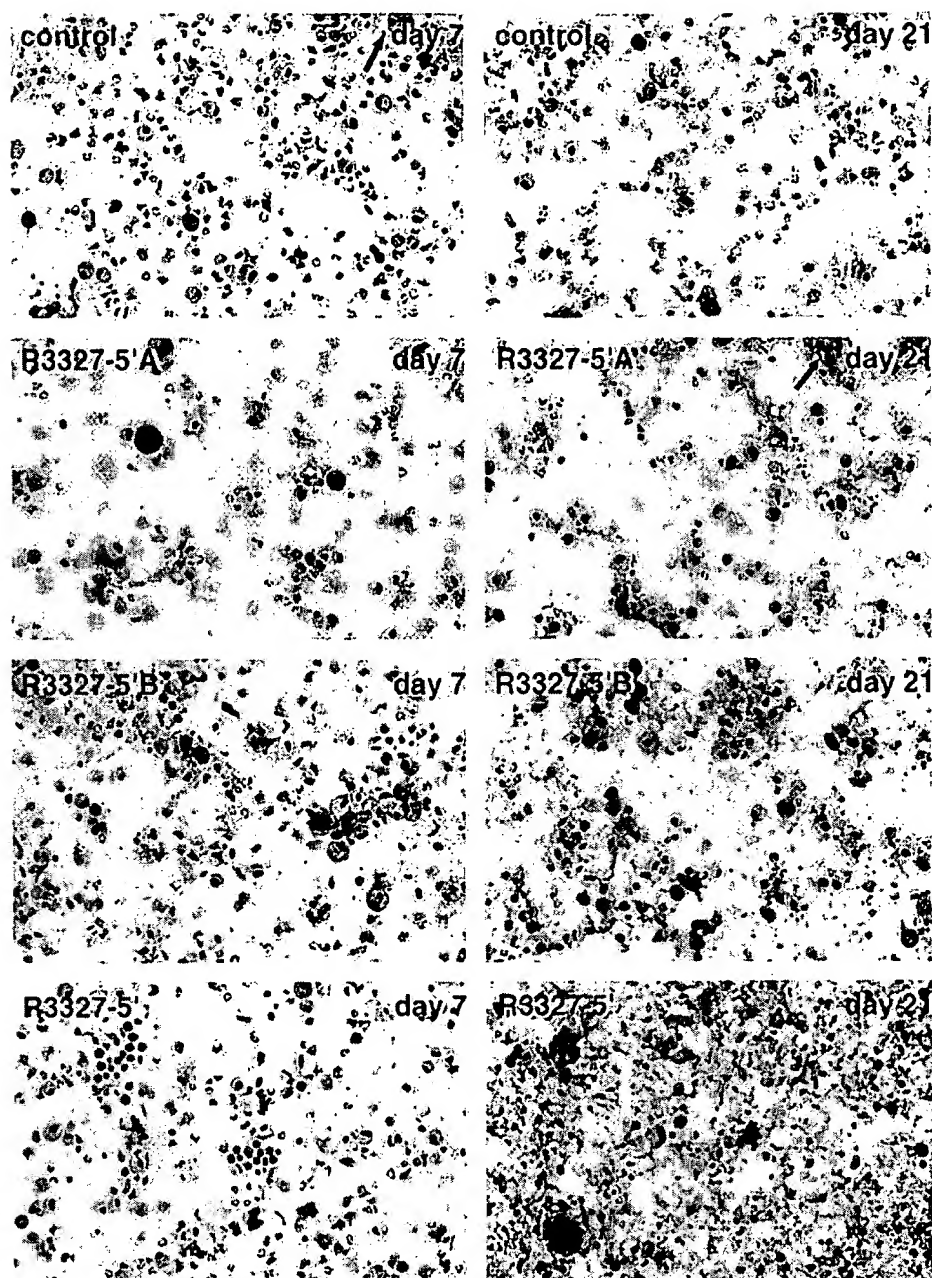
*In vitro* TGF- $\beta$  secretion by cells was measured by the previously described PAI-1-mink lung epithelial cell bioassay (13). Briefly, mink lung epithelial cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct were treated with serum-free conditioned media from R-3327-5', R-3327-5'A, and R-3327-5'B prostate cells. Each sample was assayed to determine active TGF- $\beta$ 1 concentration and then exogenously activated by heating to 80°C for 5 min to determine the total amount of TGF- $\beta$  present. Active and total TGF- $\beta$  production was assayed by adding luciferase substrate in a luminometer and measuring luminescence. All values were normalized for cell number and expressed as per nanogram DNA.

## Results

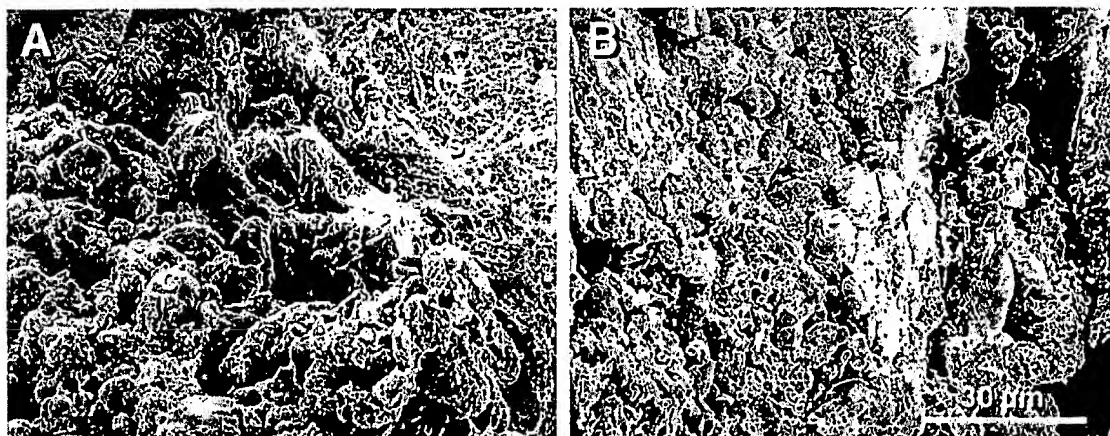
In this study, we tested the hypothesis that a weak immunological response would be associated with prostate cells that form primary tumors, and conversely, that a strong immunological response would be associated with prostate cancer cells that do not form primary tumors.

**Tumor Growth and Total Cellular Content of Control and Tumor-bearing Sponges.** The total number of cells retrieved from the sponges was determined using a hemacytometer and varied considerably with tumor cell phenotype and duration of growth in the animal (Fig. 2). As a trend, the parental R-3327-5' cell line (containing both epithelial and fibroblastic phenotypes) increased exponentially after a brief plateau and formed large (approximately 25  $\times$  20 mm) necrotic tumors. Clone R-3327-5'B-bearing sponges also showed an increase in total cell numbers during the later time points (although not as dramatic as for the parental population) and formed smaller tumors (15  $\times$  15 mm). Vascularization was observed in sponges bearing both R-3327-5' and R-3327-5'B cells. Clone R-3327-5'A (fibroblastic phenotype)-bearing sponges, however, demonstrated

a



b



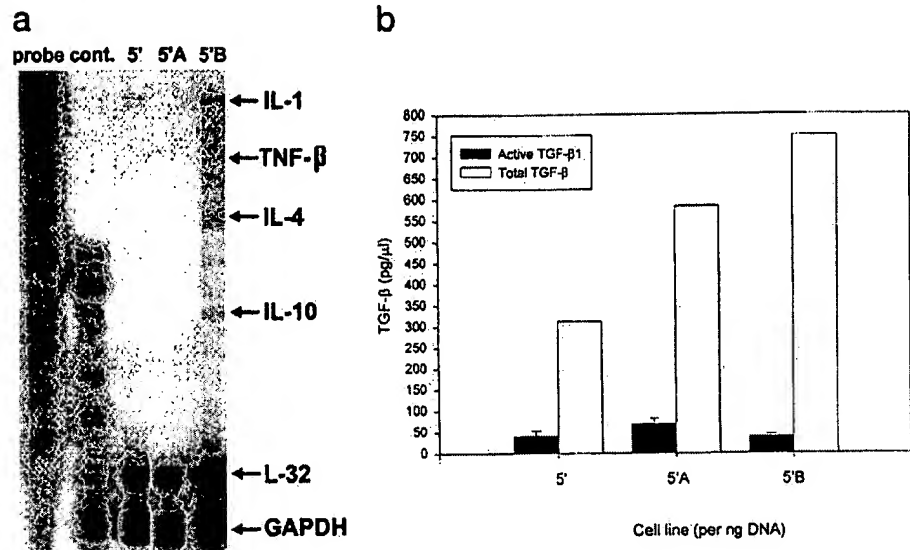


Fig. 4. *a*, cytokine mRNA production in unstimulated Dunning R-3327-5' and cloned cells. Total RNA was extracted from R-3327-5' (5'), R-3327-5'A (5'A), and R-3327-5'B (5'B) cells and assayed for cytokine gene expression using the Ribonuclease protection assay system. IL-1, IL-4, IL-10, and TNF-β mRNA are present in R-3327-5' and R-3327-5'B cells. Arrows, from top to bottom, IL-1, TNF-β, IL-4, and IL-10. Undigested probe (probe), rat control RNA (cont.), and housekeeping genes L32 and GAPDH have been included as controls. *b*, unstimulated TGF-β activity in Dunning R-3327-5' and cloned cells. Serum-free conditioned media from R-3327-5' (5'), R-3327-5'A (5'A), and R-3327-5'B (5'B) cells were assayed for active and total (active + latent) TGF-β production using the PAI-1-luciferase assay. Although R-3327-5'A cells secrete the highest levels of active TGF-β, R-3327-5'B cells secrete the highest total levels of this factor. All levels were normalized for cell number and DNA content.

an initial increase in total (all types) numbers followed by a dramatic decline in cell number, culminating in the eventual degradation of the sponge. Control sponges, containing sterile PBS, demonstrated early increases in immune cells (baseline level), which gradually declined as the sponge matrix was degraded.

**Flow Cytometric Analysis of Host Cells Recruited into the Sponges.** Characterization of the sponge-derived infiltrating leukocyte populations is summarized in Fig. 2. We targeted CD4, CD8, NK cell (NKR-P1A), and ED-2-like macrophage markers in this study. Clone R-3327-5'B- and R-3327-5'-bearing sponges showed a marked paucity in CD4, NK, and macrophage populations when compared with controls and with their R-3327-5'A-bearing counterparts. Elevated CD8 and NK populations in the R-3327-5'A-bearing sponges were detected at each time point before the sponge was degraded. Whereas macrophage populations consistently comprised the majority of the infiltrate, appreciable numbers of the other three populations, *i.e.*, CD4<sup>+</sup>, CD8<sup>+</sup>, and NKR-P1A<sup>+</sup> cells, were also retrieved by this methodology. Inflammatory response to the sponge peaked at about day 4 and then declined (data not shown); response to the tumor cells peaked at about day 29 before declining. There was no cross-reaction between any of these antibodies and tumor cells alone.

**Cytological Identification of Sponge-infiltrating Leukocytes.** At any given time point, PMN content was high in control sponges. Fig. 3a shows representative photomicrographs of the cellular presence in sponges injected with the various cell types at time points of 1 and 3 weeks. R-3327-5'A-bearing sponges maintained a relatively low total cell number throughout the experiment with a higher PMN, macrophage, and lymphocyte presence. Total cell numbers in clone R-3327-5'B and the parental R-3327-5' increased exponentially after 4 weeks, but PMN and lymphocyte numbers were never significant. The presence of polykaryotic macrophage-like cells was consistently greater in the control and R3327-5'-A bearing sponges. In these studies, differentiation between monocytes and the tumor cells was often not possible because these lines are not very conducive to stable transfection with marker proteins. This problem was resolved using mono-

clonal antibody labeling (against the ED-2-like macrophage markers) and flow cytometric analysis.

**Electron Microscopic Analysis of Tumor Development Pattern within Sponges.** Using scanning electron microscope analysis (Fig. 3b), it was demonstrated that R-3327-5'- and clone R-3327-5'B-bearing sponges displayed a considerably denser accumulation of cells, which encompassed the entire sponge. Clone R-3327-5'A-bearing sponges were characterized by the presence of intermittent foci of cells, which correlated well with the absence of primary tumor formation and eventual sponge deterioration in this line.

**Cytokine Analysis.** RNase protection assay analysis (Fig. 4a) revealed the production of detectable levels of TNF-β and IL-1, IL-4, and IL-10 mRNA by R-3327-5' and R-3327-5'B cells, although levels in the latter were much higher. R-3327-5'A cells were negative for all cytokines probed using the rCK-1 template set.

TGF-β activity analysis (Fig. 4b) in these lines revealed higher levels of active TGF-β1 in conditioned media from R-3327-5'A cells, which is consistent with previous reports of such activity in highly invasive cell lines (14). Total (latent + active) TGF-β levels, however, were highest in the R-3327-5'B line, although the active levels were the lowest. The parental R-3327-5' line, although fairly high in secreting active TGF-β1 levels, showed unexpectedly low levels of total TGF-β content when compared with the other two lines.

## Discussion

The gelatin sponge model for studying the immune response to tumor populations has been instrumental in several vital findings in mammary carcinoma systems (10, 11). The study presented here constitutes the first application of this novel methodology to the characterization of the immune response in prostate cancer. Using cloned homogeneous cell lines selected from a common heterogeneous tumor, each with differential invasive, metastatic, and tumorigenic abilities, we have developed a unique rat model system by which to probe the role of individual components of tumor populations in

Fig. 3. *a*, cytological analysis of tumor-bearing sponge populations. Cell suspensions from collagenase-digested sponges were prepared in a cytocentrifuge, fixed, and stained at 7 or 21 days after inoculation. Cell numbers in R-3327-5'A-bearing sponges increased slightly before decreasing. R-3327-5'B- and R-3327-5'-bearing sponges showed higher numbers of cells at later stages and formed large tumors. Note the lymphocyte infiltration in R-3327-5'-bearing sponges at the early stage. Saline-injected control sponges maintained a high neutrophil profile at all stages before eventual degradation. Arrows, presence of polykaryotic macrophage-like cells. *b*, scanning electron micrographs illustrating the pattern of tumor growth within the sponge implants. Note that R-3327-5'B-inoculated sponges maintained a uniform distribution of cells on the surface as well as within the sponge (B), whereas R-3327-5'A-bearing (A) sponges showed sparse and isolated colonies (c) amid the sponge matrix (s).

modifying and regulating the host immune environment for optimal tumor growth and metastatic dissemination. In this regard, the Dunning prostate adenocarcinoma of the rat provides not only the ability to study the immune response in a fully immunocompetent host, but the large body surface area of the rat also allows for the implantation of several sponges. This latter characteristic permits controls to be performed on the same animal, thus eliminating the element of immune variance among different animals.

Akporiaye *et al.* (11) have previously reported a higher host cell infiltration as well as higher total cell numbers (immune cells + tumor cells) in EMT6 mouse mammary tumor-bearing sponges. Although we have observed generally higher total numbers of cells in tumor-bearing sponges, the relative numbers of putative antitumor immune effectors was actually lower in the R-3327-5'- and clone R-3327-5'B-bearing sponges. Degradation of the sponge and eradication of clone R3327-5'A tumor cells at the site of inoculation followed an increase in NKR-P1A<sup>+</sup> and CD8<sup>+</sup> numbers, as determined by flow cytometric analysis. R-3327-5'- and clone R-3327-5'B-bearing sponges, however, resisted degradation and displayed fewer polykaryonic macrophage populations. In each case, however, there was a marked difference in the foreign-body response generated by control sponges and the response generated by tumor-bearing sponges, indicative of a tumor-specific response to the different tumor subpopulations. Cell numbers in sponges bearing these lines increased exponentially following a brief lag period and produced large tumors at the site of inoculation. This is suggestive of a modulation of the tumor micro-environment and supports previous observations of immunosuppression in tumor-bearing hosts (6–8). To our knowledge, this is the first known report of such *in vivo* immune activity in the Dunning system. Further study is needed to define this immunosuppression.

The production of immunomodulatory cytokines by several tumor cell types, including those from the prostate, has been implicated recently in tumor cell evasion of host immune defense (6). In this regard, TGF- $\beta$  is a well-characterized factor in the proliferation, differentiation, and activation of effector cells in both innate and acquired immunity (15). Effects of TGF- $\beta$  on tumor-infiltrating lymphocytes include the inhibition of growth, CTL differentiation, cytokine production, and antigen presentation. Our results indicate the presence of higher levels of active TGF- $\beta$  in the more metastatic and invasive R-3327-5'A cells, which is consistent with previous observations linking this cytokine to the invasive and metastatic phenotype (14). Additionally, IL-10, a potent inhibitor of T helper 1 (Th1)-associated cytokine secretion, is spontaneously secreted by several tumor cell types and is implicated in tumor survival by shifting the immune response to a T helper 2 (Th2) type (9). R-3327-5'B cells secrete high levels of total TGF- $\beta$ , which may be activated by several different mechanisms *in vivo*. This finding, coupled with their IL-10 secretion, suggests a potent immunosuppressive mechanism and may account for their tumorigenic ability (9). The parental R-3327-5' cells, a heterogeneous population consisting of both R-3327-5'A and R-3327-5'B cells, have unexpectedly lower total TGF- $\beta$  levels, although they secrete relatively high levels of the active form. R-3327-5' cells also produce detectable levels of IL-10 mRNA, and these intermediate levels may play a role in the ability of these cells to produce both a primary tumor and distant metastases. These preliminary studies suggest that cytokine profiles in various tumor populations may be responsible for specific aspects of immune surveillance and may be interdependent.

Loss of E-cadherin, a putative metastasis suppressor that promotes normal cell adhesion and growth, has been associated with more aggressive populations of human prostate cancer (16). Subpopulations of the Dunning R-3327-5' cells and the derived R-3327-5'B clone, but not the more invasive R-3327-5'A, express E-cadherin, which correlates well with clinical observations (3). Whether the absence of a primary tumor after R-3327-5'A injection results from a combination of strong immune rejection and absence of E-cadherin expression is an intriguing possibility that is the subject of future endeavors.

We have demonstrated the ability to retrieve viable leukocyte populations from prostate tumor-bearing sponges, which offers promise as a model for further *in vitro* and *in vivo* manipulations, and for testing adoptive immunotherapy. In doing so, we have substantiated our hypothesis and demonstrated that weaker immunological responses are associated with tumor cell populations having the ability to produce primary tumors. In a weakly immunogenic cancer, such as prostatic adenocarcinoma, the ability to study the role of angiogenic, chemotactic, or immunomodulatory factors contributing to tumor growth and maintenance recommends the sponge model system as a novel means of testing new therapeutic strategies for prostate cancer intervention.

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